Case Report

A Probable Case of Laboratory-Acquired Infection with *Salmonella typhi*: Evidence from Phage Typing, Antibiograms, and Analysis by Pulsed-Field Gel Electrophoresis

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ABSTRACT

Objective: To report a probable case of laboratory-acquired typhoid fever involving a female laboratory technologist at a major diagnostic bacteriology laboratory in Kuala Lumpur, Malaysia.

Methods: The technologist presented with clinical symptoms of typhoid fever and was admitted to a major hospital in Kuala Lumpur. *Salmonella typhi* isolated from her stools, as well as other *S. typhi* isolates she had been working with, were analyzed by Vi phage typing, antibiogram studies, and pulsed-field gel electrophoresis. The phage type and antibiograms of the isolate were identical to those of one of the laboratory strains she had been working with during her routine duties.

Results: Pulsed-field gel electrophoresis analysis of restricted chromosomal DNA confirmed the identity of the isolate with that of the laboratory isolate. The isolate involved was phage type E1 and was resistant to multiple antibiotics.

Conclusion: The results strongly suggest that the laboratory technologist acquired the infection in the laboratory in the course of her work.

Key words: *antibiogram, laboratory-acquired infection, pulsed-field gel electrophoresis, phage typing,* Salmonella typhi

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Typhoid fever remains an important public health problem in the developing world. In a rapidly developing country like Malaysia, increased urbanization has actually witnessed an increase in the incidence of cases. The causative agent of disease is *Salmonella typhi*. It is spread through food, drinks, and sometimes directly through contaminated laboratory equipment and poor laboratory practices. Laboratory-acquired infections caused by bacterial, viral, fungal, rickettsial, and parasitic agents have been recognized since the beginning of this century.[1] Laboratory workers have occasionally become infected by these microorganisms with which they are working, and some of these infections have resulted in death. For example, of the 20 typhoid deaths reported by Pike,[1] 15 occurred in Germany, 1 was reported in France, and 4 occurred in the United States. During a 33-month study in the United States by Blaser et al,[2] 24 cases, or 2.4% of all typhoid cases reported, were laboratory-acquired typhoid fever. Laboratory-associated cases of salmonellosis are also well documented in a number of published reports and surveys. For example, in Great Britain, the overall incidence of *Salmonella* infection is 0.137 infections per 1000 persons. Most of the workers affected have been microbiologists. However, in most countries endemic for *Salmonella typhi* infection, like Malaysia, reports on laboratory-acquired infections are scarce, and there have been no published reports on this. The actual risk and incidence of laboratory-acquired infection is difficult to measure, but is bound to be significant as the laboratory workload increases as a result of increased disease activity. Also, surveillance data on laboratory-associated infections are difficult to obtain, because the infections are often subclinical and have an atypical incubation period and route of infection. Moreover, laboratory directors may not report any laboratory-related incidents for fear of reprisal or embarrassment. This report presents a probable case of laboratory-acquired typhoid fever involving a laboratory technologist in Kuala Lumpur, Malaysia.

The laboratory technologist involved (36-yr-old female) is employed at a major diagnostic bacteriology laboratory in Kuala Lumpur, Malaysia. She showed clinical symptoms of typhoid fever and was admitted to the

Kuala Lumpur Hospital. *Salmonella typhi* was isolated from a stool specimen, and was maintained and identified by standard methods.[3] The patient was treated with antibiotics and subsequently made an uneventful recovery, after 2 weeks of therapy. Unfortunately, a detailed clinical report of the patient's symptoms was not available. Subsequently, *S. typhi* isolates from this individual and other *S. typhi* isolates (which had been handled by this technologist at the time of the incident) were analyzed using Vi phage typing, antibiotic sensitivity testing, and pulsed-field gel electrophoresis (PFGE). Vi phage typing of the isolates was performed according to standard procedures by the Salmonella Reference Centre at the Institute for Medical Research, Kuala Lumpur. Repeated subculturing of isolates was avoided and stocks of the primary isolates were maintained at −70°C. *Salmonella typhi* isolates were tested for sensitivity to ampicillin, chloramphenicol, tetracycline, kanamycin, sulfamethoxazole, trimethoprim, carbenicillin, ciproxozime, and nalidixic acid by standard disk-diffusion procedures to measure resistance, according to National Committee for Clinical Laboratory Standards (NCCLS) guidelines. Genomic DNA for PFGE analysis was prepared by the method previously described.[4,5] The intact chromosomal DNA was then digested with four restriction enzymes, *Xba*I (5′-TCTAGA-3′), *Spe*I (5′-ACTAGT-3′), *Avr*II (5′-CCTAGG-3′), and *Not*I (5′-GCGGCCGC-3′) (New England Biolabs, Beverly, MA), as previously described.[4] Restricted DNA fragments were then separated by PFGE, using the contour-clamped homogeneous electric field (CHEF) method on a CHEF DR-II or DR-III system (Bio-Rad Laboratories, Richmond, CA) in gels of 1% agarose in 0.5X TBE buffer (0.1 M Tris, 0.1 M boric acid, 0.2 mM EDTA) for 24 hours at 200 V at a temperature of 14°C, with ramped pulsed times varying according to the enzymes used (ranging from 1 to 50 sec). Gels were stained with ethidium bromide and photographed with an ultraviolet transilluminator (Spectroline Co., Westbury, NY, USA, 302 nm). The DNA size standards used were a lambda ladder consisting of concatemers and MidRange II PFG Marker (New England Biolabs, Beverley, MA, USA).

The phage types and antibiograms of the isolate from the infected technologist were identical to those of the laboratory strains (Table 1). Both the isolates were of phage type E1 and showed multiple resistance to ampicillin, chloramphenicol, tetracycline, kanamycin, sulfamethoxazole, and trimethoprim. The isolate was obtained from the laboratory technologist 12 days after the suspected laboratory isolate was cultured in the laboratory. Except for these two isolates, all the other isolates analyzed (more than 100 isolates) were sensitive to the antibiotics tested (data not shown). Pulsed-field gel electrophoresis analysis following digestion of chromosomal DNA with the *Xba*I, *Spe*I, *Avr*II, and *Not*I showed that the two isolates were identical (see Table 1). Two DNA profiles obtained with *Xba*I and *Spe*I are shown in Figure 1. Stable and reproducible PFGE patterns comprising 15 to 24 fragments were obtained with the four restriction endonucleases.

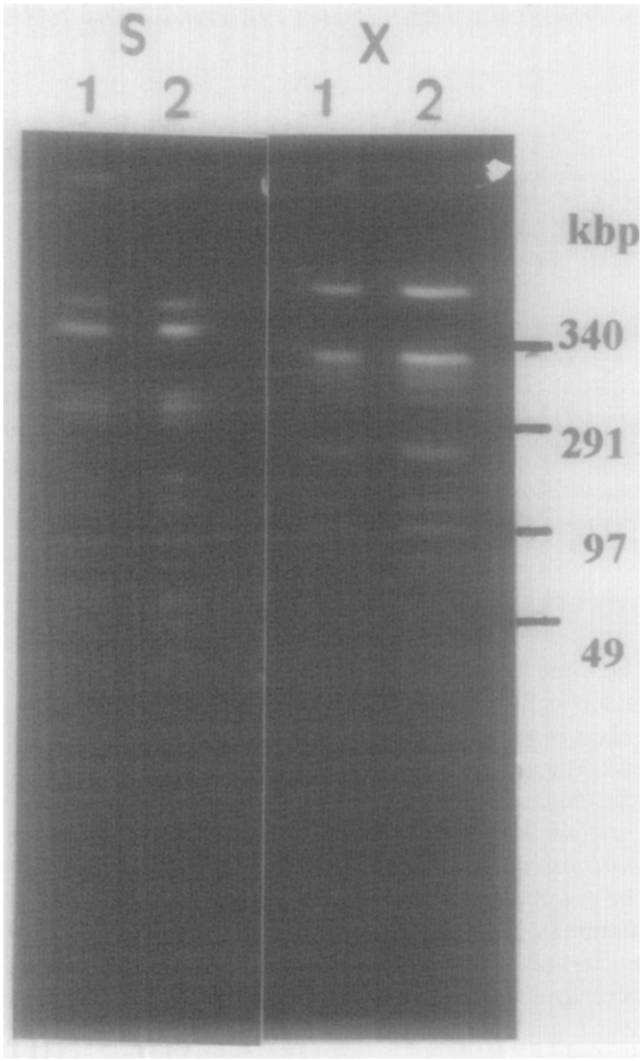
The history of laboratory-acquired typhoid fever in the United States has been well documented since 1915.[2] Typhoid fever has accounted for more reported deaths than any other laboratory-acquired infection.[1] However, such documentations are not available in Malaysia and other developing countries. Because of the higher risk of contamination with the infectious agent to the personnel directly involved in the laboratory work, the laboratory can be a significant reservoir of *S. typhi*.[1] The most common routes of infection include ingestion of microorganisms through mouth pipetting, transfer of organisms to the mouth from contaminated items such as pencils or fingers,[6] consumption of food and drinks in the laboratory,[7] and accidental splashes that fall into the mouth. Specimen collection, processing, and manipulation of cultures during routine laboratory operations frequently contaminate containers and bench top equipment through the generation of aerosols.[8]

Some further observations can be made from the results of the present study. First, of the many *S. typhi* isolates processed by the technologist in the course of her work, the one that was transmitted to her was a strain possessing multiple resistance to several antibiotics. A similar case of laboratory-acquired typhoid fever in Malaysia involved a graduate student working with antibiotic-resistant *S. typhi* (unpublished observation). In contrast, another laboratory working with hundreds of nonresistant *S. typhi* has not had an incident of laboratory-acquired infection. This suggests that antibiotic-resistant *S. typhi* was more virulent or infective and that a lower infectious dose is required to cause disease. The suggestion that antibiotic-resistant *S. typhi* is more virulent than endemic strains has been made previously.[9] Although

Table 1. Phage Types, Antibiograms, and PFGE Patterns of *S. typhi* Isolates from the Laboratory Technologist and the Suspected Laboratory Isolate

Isolate	*Date of Isolation*	*Source*	*Phage Type*	*Antibiogram**	*PFGE Pattern†*
Suspected laboratory isolate‡	9-10-92	Blood	E1	RRRRRRSSS	X1S1A1N1
Laboratory technologist	9-22-92	Stool	E1	RRRRRRSSS	X1S1A1N1

*Resistance (R) or sensitivity (S) to the following antibiotics (in order): ampicillin, chloramphenicol, tetracycline, kanamycin, sulfamethoxazole, trimethoprim, carbenicillin, ciproxozime, and nalidixic acid.
†PFGE pattern profile of restricted DNA fragments (arbitrarily assigned as patterns 1,2,3,... etc.). X = *Xba*I; S = *Spe*I; A = *Avr*II; N = *Not*I.
‡Isolate originally obtained from a 26-year-old male.



strictly anecdotal, these observations would imply that extra care should be exercised when working with antibiotic-resistant strains of *S. typhi* in the laboratory. In addition, the present study also emphasizes the value of molecular techniques, such as PFGE,[10] in investigating the origins and transmission of pathogenic bacteria. Given the fact that clinical isolates of *S. typhi* show considerable genetic diversity,[11] the absolute identity (by PFGE) of the two isolates in this particular case report has more definitively identified the origin of the infecting strain.

The present study also reiterates the fact that laboratory personnel can be at increased risk of being contaminated with *S. typhi*. The paucity of data on laboratory-acquired enteric infection in this country does not imply that this problem is uncommon. A laboratory worker is constantly being exposed to the pathogen, and control of laboratory infection must be based on strict adherence to standards for laboratory safety. There is an urgent need to consolidate efforts to document all cases of *Salmonella* infection, including laboratory-acquired infections. Such surveillance data are necessary for better and more effective control of this important human pathogen.

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